

THE AMENDMENTS

In the Specification:

Amend the paragraph starting at page 17, line 25:

Marker molecules for use according to the present invention may comprise one or more markers chosen from p13.5, p14, p15, p16 (also referred to p16^{INK4a}, ~~SEQ ID NO: 13~~), p19, p21, p27, p53, pRb, p14ARF (~~SEQ ID NO: 14~~), cyclin A, cyclin B, cyclin E, MDM-2, MCM2, MCM5, MCM6, CDC2, CDC6, Id1, osteopontine, GRP, renal dipeptidase, her2/neu, TGFβII receptor, HPV associated markers e.g. derived from HPV genes L1, L2, E1, E2, E4, E5, E6 or E7, etc. A selection of markers useful in one embodiment of the present invention for the detection of medically relevant conditions is shown below in Table 1.

Amend Table 1 at page 21:

marker for	cell type	antigen	Antibody	supplier	Literature
cell type	epithelial cells	human epithelial cell surface glycoprotein	HEA125 IgG1 (W, IHC, ICC, IF)	Research Diagnostics Inc.	Kommoss et al., Hum Pathol. 2000 Sep;31(9):1055-61
		Human epithelial proliferation 40 kD protein (from LoVo)	AUA-1 IgG1 (Elisa)	Research Diagnostics Inc.	Gottschalk et al, Pathol Res Pract. 1992 Feb;188(1-2):182-90
		Human epithelial antigen (34+39 kD)	Ber-EP4, IgG1 (IHC, Elisa)	Dako	Latza U et al., J Clin Pathol. 1990 Mar;43(3):213-9
		Human epithelial proliferating antigen (40 kD)	AUA-1 (Elisa, W, IHC)	Research Diagnostics Inc.	Epenetos, A et al., Lancet. 1982 Nov 6;2(8306):1004-6
	endocervix columnar cells	Cytokeratin 18 (45 kD) SEQ ID NO: 9	RGE 53, IgG1 (W, IHC, IF)	Research Diagnostics Inc.	Smedts F et al., Am J Pathol. 1990 Mar;136(3):657-68
		Cytokeratin 18 (45 kD)	RCK 106 (W, IHF, IHC)	Research Diagnostics Inc	Smedts F et al., Am J Pathol. 1990 Mar;136(3):657-68

		Cytokeratin 8 (52.5 kD) SEQ ID NO: 8	CAM 5.2 (W, IHC)	BD PharMingen	Smedts F et al., Am J Pathol. 1990 Mar;136(3):657-68
	Endocervical columnar cells	Mucin Antigens (Tn, STn, MUC1, MUC2	DF3	Centocor	Tashiro et al., Hum Pathol. 1994 Apr;25(4):364-72
	Endocervic Columnar cells	Concanavalin A receptor			Herckenrode et al., Br J Cancer. 1988 Mar;57(3):293-4 ; Koch et al., Br J Cancer. 1986 Jan;53(1):13-22
	Endocervix	GalNacTransferase Oligosaccharyltransferase			Chilton et l., Endocrinology. 1988 Sep;123(3):1237-44

Amend Table 2 starting at Page 28, line 1:

Alpha-1 Catenin (Swissprot Accession P35221; also known as Cadherin-associated protein Alpha E-Catenin) SEQ ID NO: 4	Squamous epithelia	High in normal cervical epithelium at cell-cell- boundaries Strong reduction in high grade SILS	de Boer et al., 1999, Am J of Pathol, 155:505-515
Alpha-2 Catenin (Swissprot Accession P26232; also known as Alpha-Catenin related protein Alpha N-Catenin) SEQ ID NO: 5			
beta-Catenin (Swissprot Accession P35222 also known as PRO2286) SEQ ID NO: 6	Squamous epithelia	High in normal cervical epithelium at cell-cell- boundaries Strong reduction in high grade SILS	de Boer et al., 1999, Am J of Pathol, 155:505-515
Desmoplakin (Swissprot Accession P15924; also known as DP 250/210 kDa paraneoplastic pemphigus antigen)	stratified epithelia, simple epithelia, including glands, urothelium, thymic reticular epithelium, hepatocytes, intercalated disks of	↓ in HSIL area	de Boer et al., 1999, Am J of Pathol, 155:505-515

	myocardium and arachnoid cells of meninges suprabasal layers of cervix (Superficial cells largely negative)		
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Amend Table 3 at Page 29:

Table 3

Marker	Histological testing	Cytological testing	Western Blot Analysis (clinical samples were freshly lysed with MTM buffer)
E-Cadherin (Swissprot Accession P12830; also known as Uvomorulin, Cadherin-1, CAM 120/80; e.g.epitope: C- Terminus; AA735- 883) SEQ ID NO: 3	Squamous epithelia, (Parabasal, intermediate cells) no columnar epithelia	Parabasal, intermediate cells, no columnar cells	Only weak signal for HT- 29. All clinical samples are negative
p120 Swissprot Accession O60716; p120 catenin, p120(ctn), Cadherin- associated Src substrate, CAS, p120(cas); e.g.epitope: C-Terminus; AA790- 911) SEQ ID NO: 42 8	Squamous epithelia, (Parabasal, intermediate cells) also very strong in columnar epithelia	Very strong staining of parabasal, intermediate cells strong columnar cells	Only negative control (lymphocytes) and positive control (C4.1) positive
gamma-Catenin Swissprot Accession Q86W21; also known as Plakoglobin; e.g. epitope: C-Terminus; AA553-738) SEQ ID NO: 1	Squamous epithelia, (Parabasal, intermediate cells) no columnar epithelia, total epithelium is stained indysplasia	Very strong staining of parabasal, intermediate cells no columnar cells	Double bands (82/95 kD) in 60 % of samples (9/15); after acetone precipitation of 150 µl of samples: 87% (13/15) positive
Ep-Cam (Tumor-associated calcium signal transducer 1, Swissprot Accession P16422; also known as	strong columnar epithelia, at very high concentrations rather unspecific (cytoplasmic) staining	strong columnar cells, at very high concentrations rather unspecific (cytoplasmic) staining of squamous epithelia,	

Major gastrointestinal tumor-associated protein, GA733-2, Epithelial cell surface antigen, Epithelial glycoproteins, EGP, Adenocarcinoma-associated antigen KSA KS 1/4 antigen Cell surface glycoprotein Trop-1) SEQ ID NO: 2	of squamous epithelia, (Parabasal, Intermediate Cells)	(Parabasal, Intermediate Cells)	
Involucrin (Swissprot Accession P07476) SEQ ID NO: 7	Strong staining of squamous epithelia, (Parabasal, Intermediate Cells) and columnar epithelia; unspecific staining of stromal cells;	All cells and structures positive	

Amend the paragraph starting at page 37, lines 13:

In order to evaluate markers indicating the adequacy of cervical swabs, cervical sections (fixed in 4 % formaldehyde solution and paraffin-embedded) were stained with antibodies directed against Cytokeratin 18 (marker for endocervical columnar epithelia) and Cytokeratin 10/13 (~~SEQ ID NO: 10/11~~, marker for ectocervical squamous epithelia). Figure 1 shows specific staining of endocervical epithelia with anti-Cytokeratin 18 antibody and specific staining of ectocervical epithelia with anti-Cytokeratin 10/13 antibody. The experiment was performed as follows:

Amend the paragraph starting at page 40, line 9:

Western blot analysis was performed as follows: Patient samples were collected with a cervical brush and directly lysed in Laemmli Sample Buffer (2% SDS, 60mM Tris pH.6.8, 0.01%, 100 mM DTT) for 5 min at 95°C (1×10^7 cells/ml) with subsequent sonification (5x5sec pulses, maximum intensity). Lysates were centrifuged for 12 min at 16,600xg in a microcentrifuge and supernatant was transferred into a new tube. Precast 4-20% linear gradient ~~Acrylamide~~ acrylamide gels (Criterion System, Bio-Rad) were loaded with 10µl (10^5 cells) of

whole cell extracts and proteins were separated at 25mA constant current for 45 min. Proteins were transferred from the gel to ~~Hybond~~ HYBOND[®] (Hydrophobic polyvinylidene difluoride membrane) ECL Nitrocellulose membrane (Amersham) by standard tank blotting using the Bio Rad Criterion Blotter (15 min at constant 100 Volt and subsequently 45min at constant 50 Volt). Nitrocellulose-membrane was stained for 5 min in Ponceau S solution to assure protein transfer. Ponceau S solution was removed by 2x10 min washes in PBS. For immunodetection, blots were blocked over night in blocking buffer (10% milk powder in PBS with 0.1% TWEEN[®]-20). Primary antibodies were incubated at dilutions according to the manufacturer in blocking buffer for 1 h at RT with agitation (CK18: MAB 3236), 1:1000, CHEMICON; CK 10/13: DE-K13, 1:500, DAKO, p16^{INK4a}: D7D7, 1:140, MTM Laboratories). After 6 washes for 10 min with PBS/0.1% TWEEN[®]-20, blots were incubated with rabbit anti mouse-HRP, (DAKO, diluted 1:5,000 in blocking buffer) for 1 h at RT. After 6 washes for 10 min with PBS/0.1% TWEEN[®]-20, membranes were incubated for 5 min in substrate solution (Super Signal West Femto Maximum Substrate, Pierce), wrapped in a plastic envelope and exposed to an x-ray film for 1-5 min. Finally, x-ray films were developed, fixed, dried and documented with an imaging system (Bio-Rad). The same samples were used to perform ELISA analysis for p16^{INK4a}, CK 10/13, CK18. The detected signals and results were the similar to the Western blot analysis and the same conclusions were drawn.

Amend the paragraph starting at page 41, line 3:

The ELISA analysis was performed as follows: Flat bottom 96 well plates (~~MaxiSorb~~ MAXISORB[®], chemical product; Nunc) were coated with capture antibody (p16^{INK4a}: MTM-E6H4, 2µg/ml in PBS, MTM Laboratories; CK10: MS481P1ABX, 2µg/ml, dianova; CK18: K18.7, 2µg/ml, dianova; 50µl/well) over night at 4°C. Plates were washed 6x with PBS/0.1% ~~Tween-20~~ TWEEN[®]-20 and blocked with ~~Superblock~~ SUPERBLOCK[®] buffer (chemical reagent, Pierce). Solubilized protein extract from cervical swabs were dissolved in incubation buffer (PBS, 3% SUPERBLOCK[®] (Chemical reagent), 0.1% TWEEN[®]-20), and added in triplicates to each well. After 1 h incubation at RT, plates were washed 6x with PBS/0.1% TWEEN[®]-20 and incubated with biotinylated detection antibody (p16^{INK4a}: MTM-D7D7 (0.2 µg/ml, MTM Laboratories, CK10: MS481-BO, 200 µg/ml, dianova; CK18: MS142-BO, 200 µg/ml, dianova;

in incubation buffer) for 1 h at RT. Following 6x washes with PBS/0.1% ~~Tween-20~~ TWEEN[®]-20 TMB, 50 µl of Streptavidin-coated Alkaline Phosphatase (1:1000 dilution; Dianova) was added for 30 min. Thereafter, plates were washed 6x with PBS/0.1% TWEEN[®]-20 and 100 µl of p-nitrophenyl phosphate substrate (PnPP; dissolved in diethanol amine buffer) were added to each well. OD 405 nm (620 nm reference wavelength) was measured with an ELISA reader (TECAN[®]) after 30 min, 1h and 2 hrs. The present example shows, that the sandwich ELISA format exhibits sensitivity, which is suitable for the use in the methods according to the present invention. For use in the method disclosed herein the sandwich ELISA format as described in this example may be applied to multiple marker molecules, such as markers for normalization/adequacy and markers characteristic for medically relevant conditions.

After page 50, insert the attached sequence listing pages 1-27.